



# Genome organization and transcription of arteriviruses

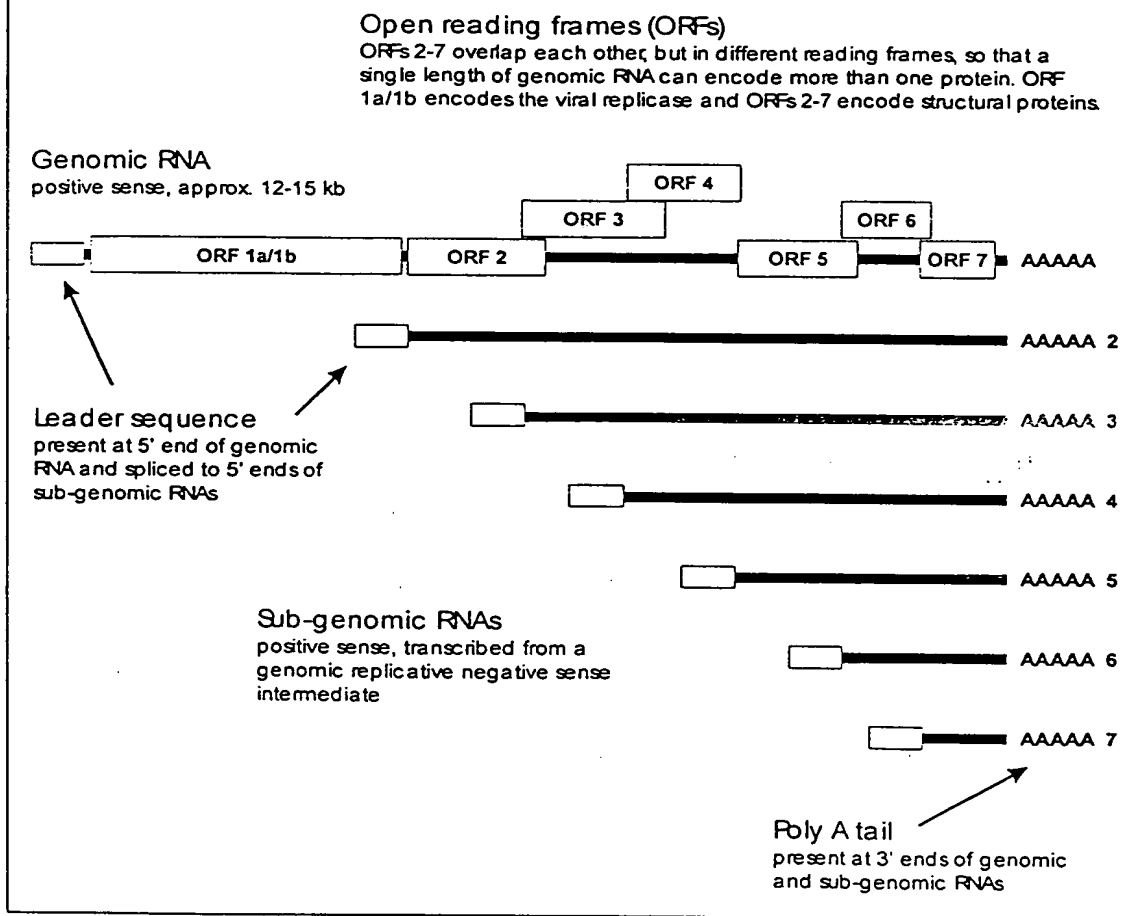


Fig. 1:

Schematic diagram of the genomic organization and transcriptional strategy of the family *Arteriviridae*.



## Construction of a vector expressing the neutralization determinant of the viral large glycoprotein

The N-terminal hydrophilic ectodomain (amino acids 1-121) of the G(L) envelope glycoprotein contains the neutralization domains of EAV (Balasuriya et al., 1997, Virology 232, 114-128). The corresponding coding region of the viral ORF 5 (nucleotides 1-363) was inserted into the mammalian expression vector pcDNA3.1/His.

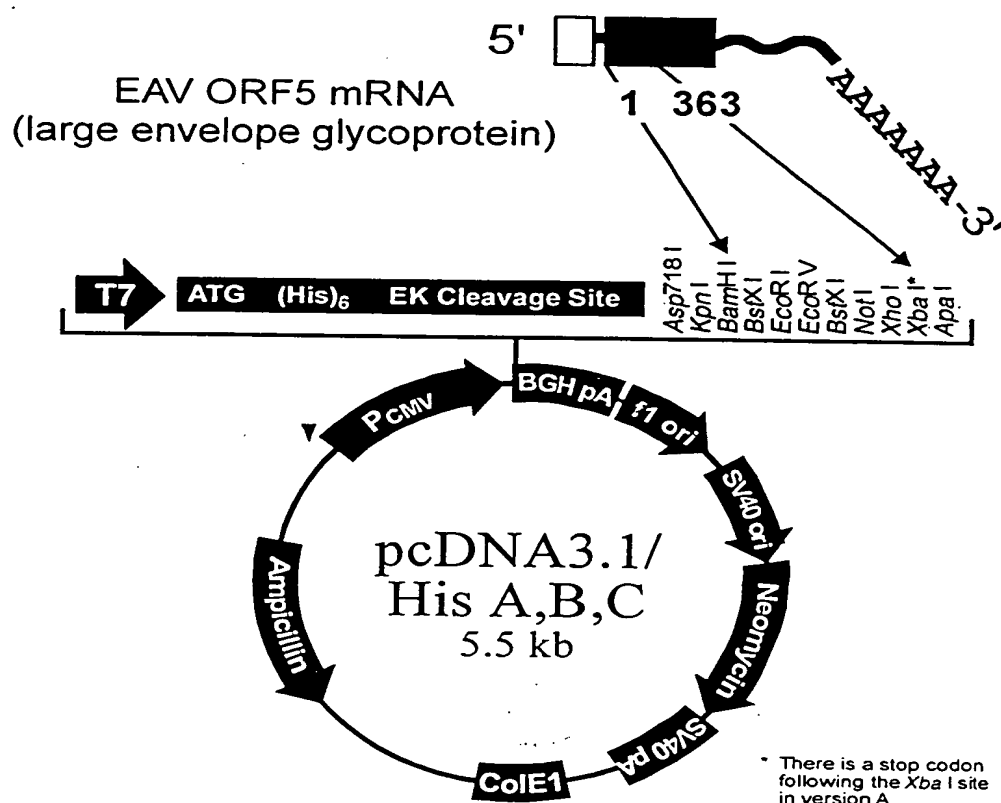
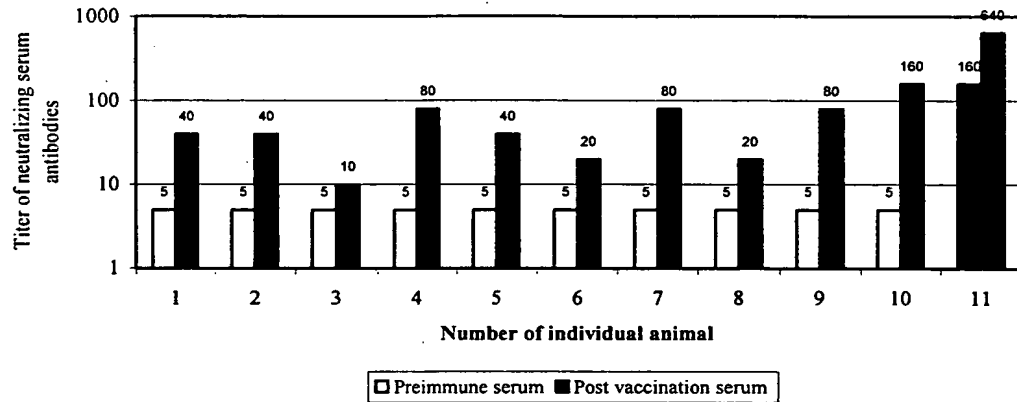


Fig. 2:

Schematic diagram of the strategy used for molecular cloning of neutralizing domain of equine arteritis virus (EAV). A part of the cDNA of viral ORF5 expressing the N-terminal hydrophilic ectodomain of the EAV envelope large glycoprotein was inserted into the corresponding sites of mammalian expression vector pcDNA3.1.

A

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmid pCR3.1-EAV-O5-BX-C14 expressing ORF 5 of equine arteritis virus



B

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmid pCR3.1-EAV-O5-BX-C14 expressing the ORF 5 of equine arteritis virus

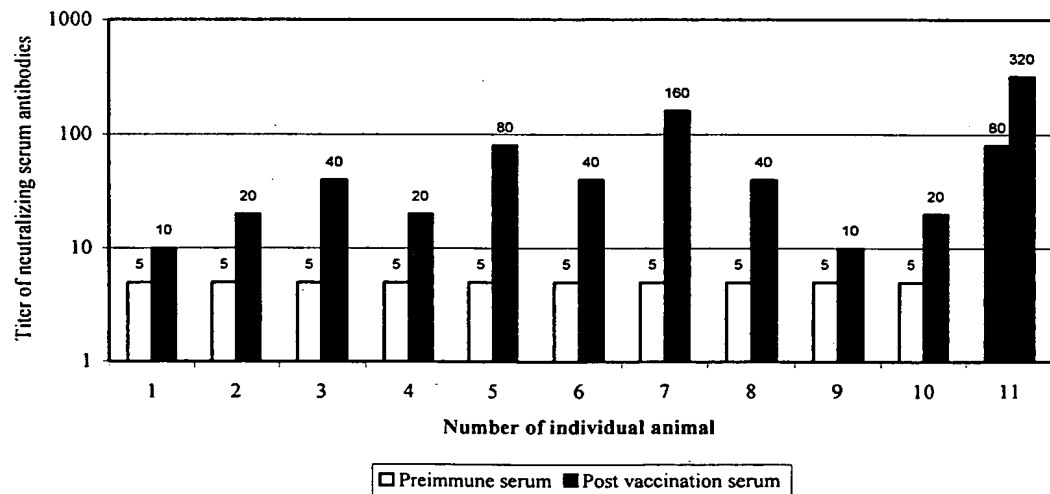
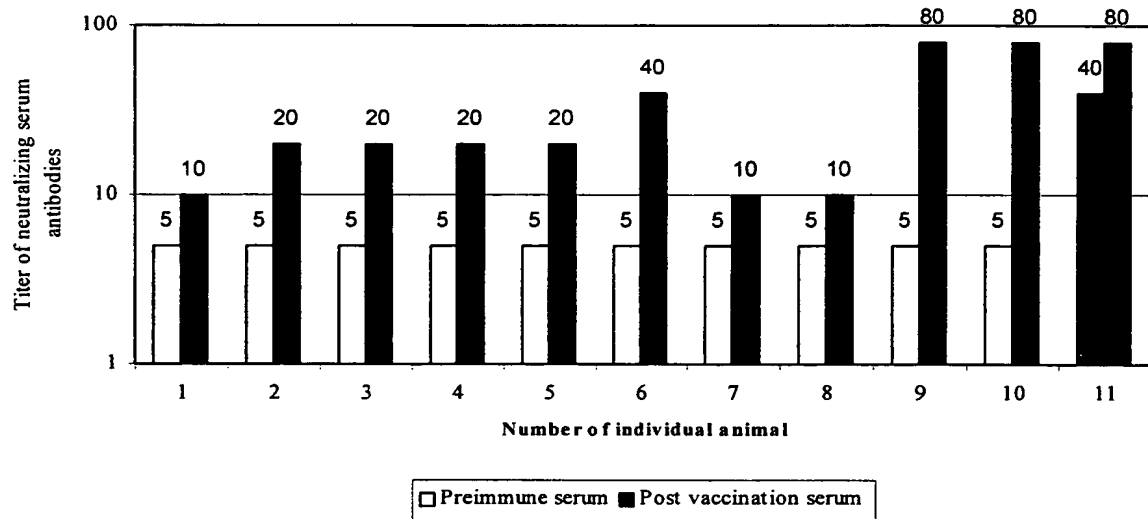


Fig. 3:

The results of neutralization tests with recombinant plasmid pCR3.1-EAV-O5-BX-C14. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

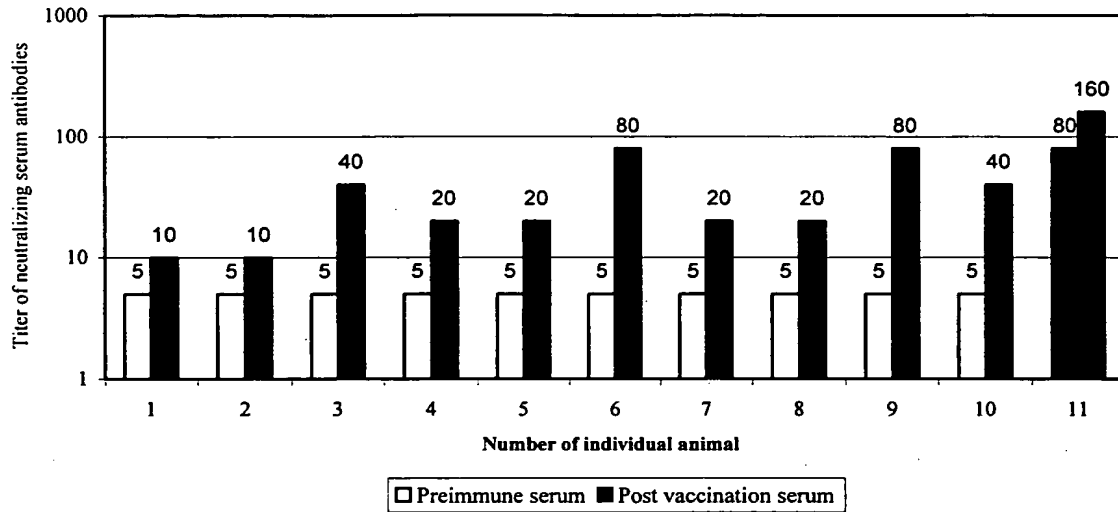
Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O7-BX-C3 expressing ORF 5 and 7 of equine arteritis virus



**Fig. 4:**

The results of neutralization tests with recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O7-BX-C3. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

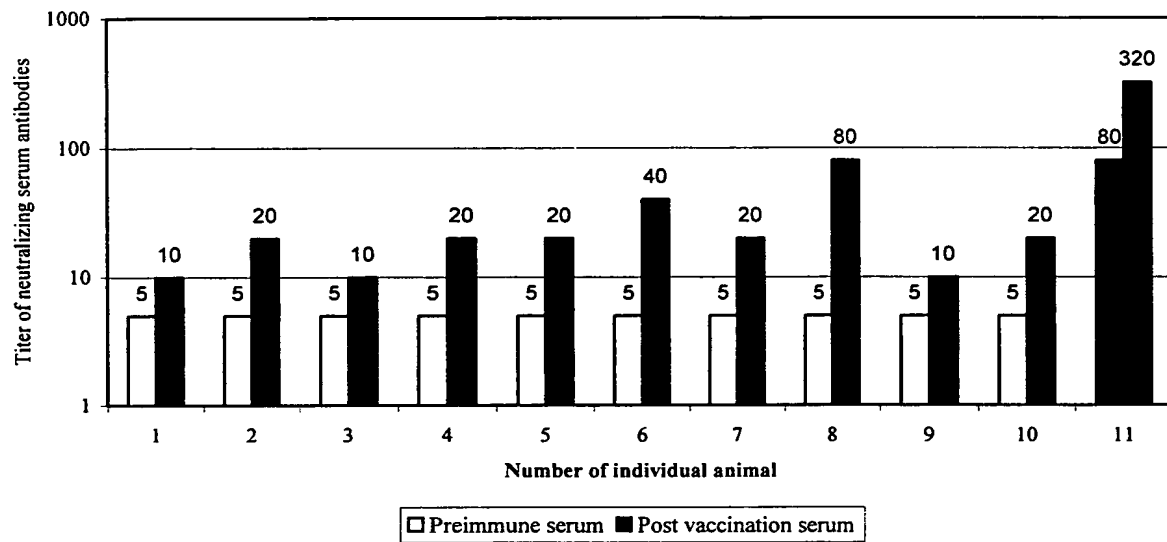
Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pDP-EAV-O7-BgS-C2 and pDP-EAV-O5-BgS-C1 expressing ORF 7 and 5 of Equine arteritis virus, as well as pWS2ms expressing IL-2 gene



**Fig. 5:**

The results of neutralization tests with recombinant plasmids pDP-EAV-O5-BsS-C2 and pDP-EAV-O7-BsS-C1. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

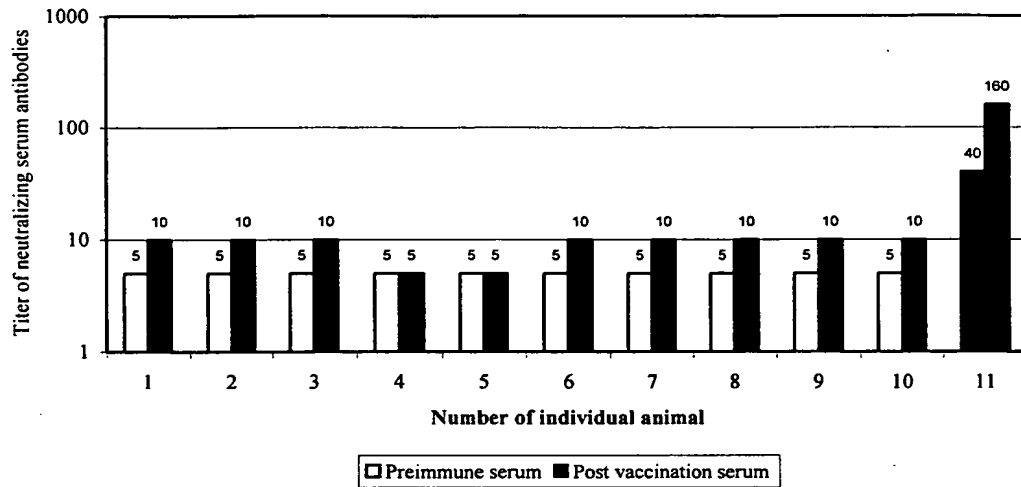
**Diagram presenting the data of DNA immunization of Balb/c mice  
with recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O6-BE-C4  
expressing ORF 5 and 6 of equine arteritis virus**



**Fig. 6:**

The results of neutralization tests with recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O6-BE-C4. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

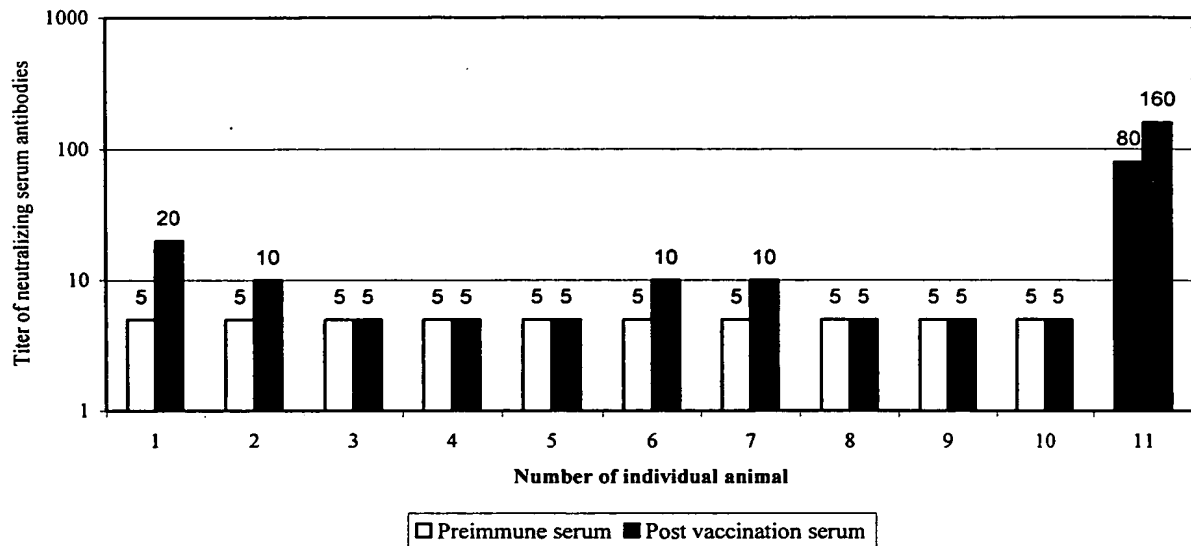
**Diagram presenting the data of DNA immunization of Balb/c mice  
with recombinant plasmids pCR3.1-EAV-O3-BX-C1  
expressing ORF 3 of equine arteritis virus**



**Fig. 7:**

The results of neutralization tests with recombinant plasmid pCR3.1-EAV-O3-BX-C1. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

**Diagram presenting the data of DNA immunization of Balb/c mice  
with recombinant plasmids pCR3.1-EAV-O4-BX-C3  
expressing ORF 4 of equine arteritis virus**

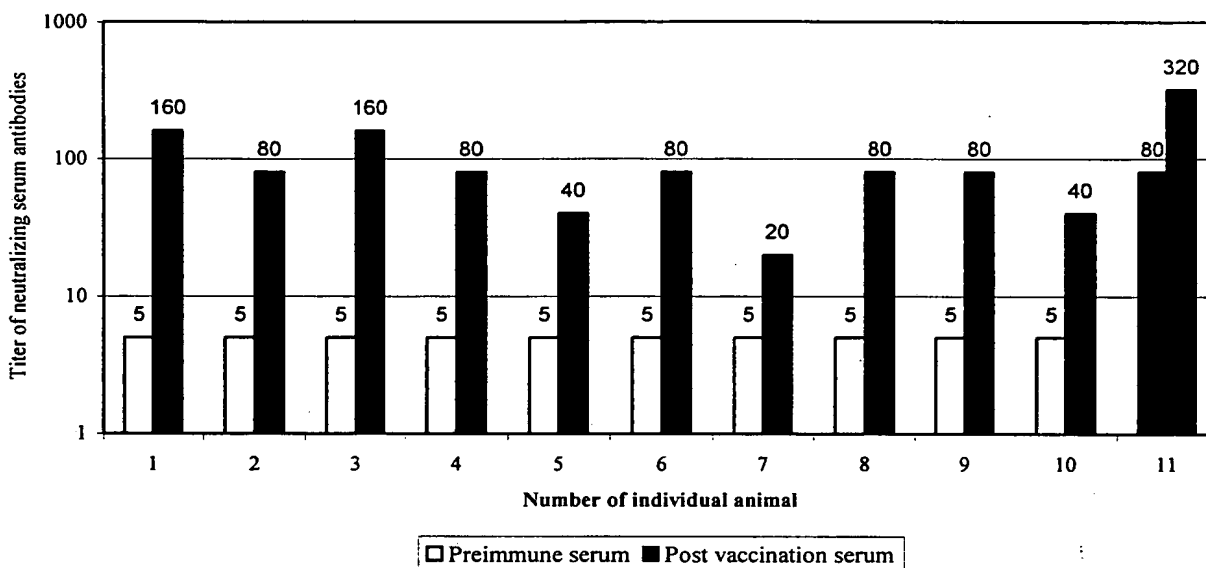


**Fig. 8:**

The results of neutralization tests with recombinant plasmid pCR3.1-EAV-O4-BX-C3. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.



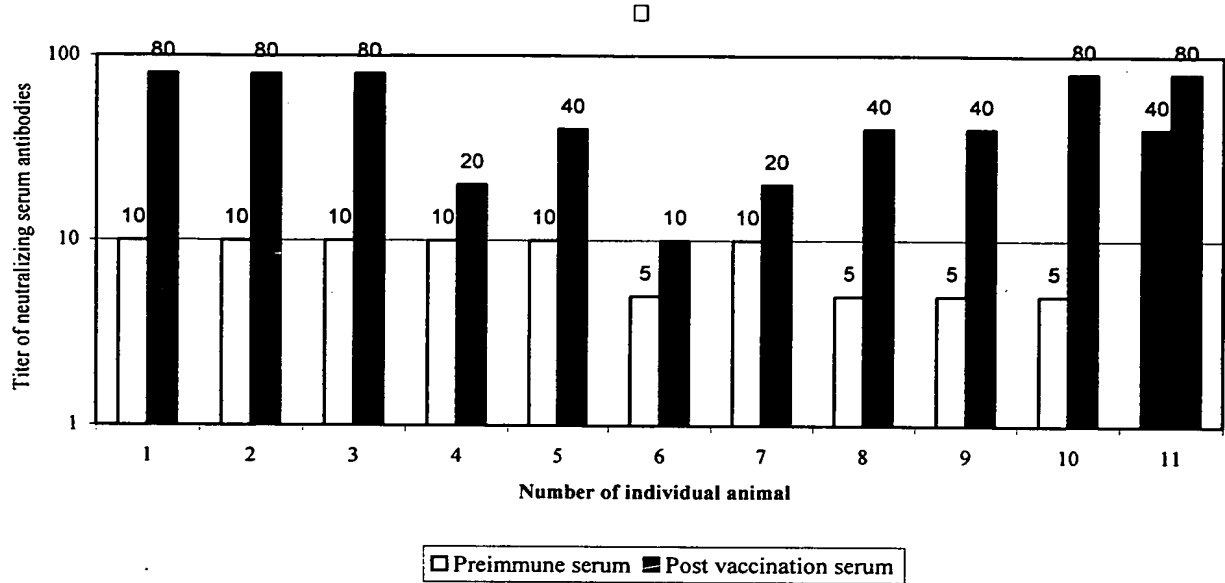
**Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmid pC3.1-EAV-O5-del-121 expressing the amino terminus (aa 1-121) of ORF 5 of equine arteritis virus**



**Fig. 9:**

The results of neutralization tests with recombinant plasmid pCR31-EAV-O5-del-121. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

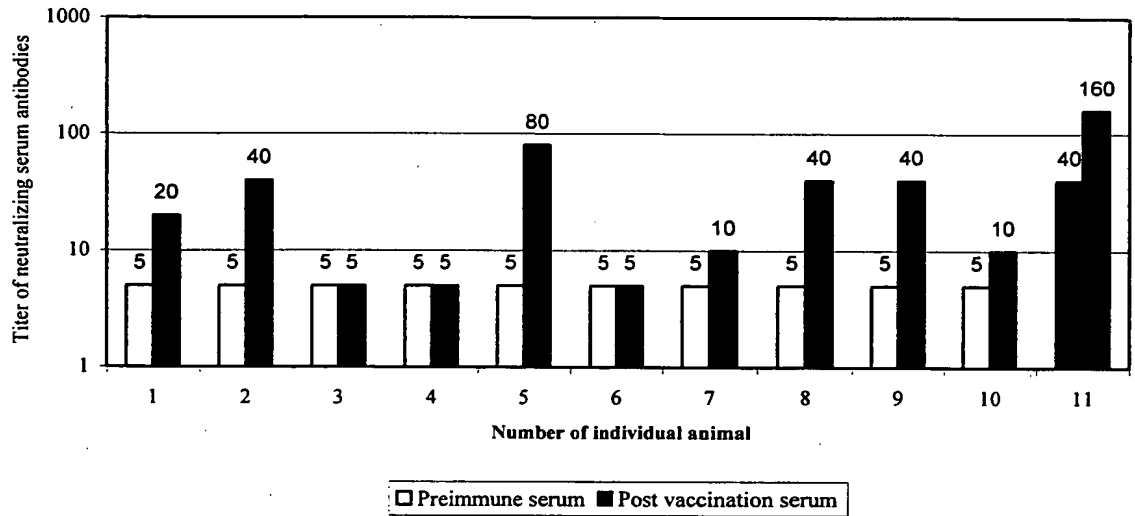
Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pCR3.1-EAV-O2-BX-C5, pCR3.1-EAV-O5-BX-C14, and pC31-EAV-BE-O6 expressing ORFs 2, 5, and 6 of EAV, as well as pWS-2ms-C1 expressing IL-2



**Fig. 10:**

The results of neutralization tests with recombinant plasmids pCR3.1-EAV-O2-BX-C5, pCR3.1-EAV-O5-BX-C14, and pCR3.1-EAV-O6-BE-C4. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

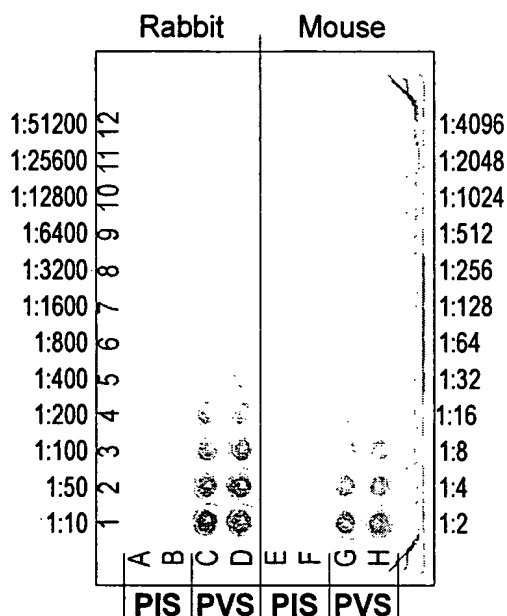
Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pCR3.1-EAV-O2-BX-C5 and pCR3.1-EAV-O4-BX-C3 expressing ORF 2 and 4 of equine arteritis virus



**Fig. 11:**

The results of neutralization tests with recombinant plasmids pCR3.1-EAV-O2-BX-C5 and pCR3.1-EAV-O4-BX-C3. The individual animals are indicated with number 1 to 10. The column 11 served as positive control of a New Zealand white rabbit immunized with inactivated EAV.

# Development of an ELISA system for the detection of specific antibodies directed against equine arteritis virus antigens



## **Antibodies conjugated with horse-radish peroxidase:**

Anti-rabbit IgG-POD 1:3000  
 Anti-Mouse IgG-POD 1:3000  
 (Boehringer Mannheim)

## **Substrate:**

Chromogen TMB (Behring)

**PIS** Preimmune serum

**PVS** Post vaccination serum

**Fig. 12:**

An example of an ELISA for the detection of EAV specific antibodies. Microtiter plates were coated with EAV Protein over night at room temperature . The assay was stopped after 30 min and read according to standard procedures.